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Genetically modified soybean in a goat diet: Influence on kid performance

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ABSTRACT

The *in vivo* and *post mortem* performance and serum immunoglobulin G (IgG) concentration in kids born from goats fed conventional (group C) or genetically modified (group T) soybean meal were evaluated. The goat colostrum quality, in terms of chemical composition, as well as immunoglobulin concentration, and the presence of feed DNA fragments were also investigated. Kid birth weights were similar, while significantly ($P < 0.05$) higher in those born from goats in group C at day 30 and at slaughtering. In addition, kids from mothers fed conventional soybean recorded significant ($P < 0.05$) higher height at the withers and chest width. Concerning the *post mortem* measurements, only carcass weights were significantly affected by the treatment resulting in lighter T kids ($P < 0.05$). Colostrum from the treated groups recorded a significantly ($P < 0.01$) lower percentage of protein and fat. Similarly, both chemical parameters significantly differed in milk collected 15 days after kidding, although these differences disappeared in the successive samplings. Both colostrum and kids serum IgG concentration were significantly ($P < 0.01$) lower in the treated groups. Transgenic target DNA sequences (35S and CP4 EPSPS) were not detected in colostrum from goats that received a diet containing conventional soybean meal. By contrast, transgenic DNA fragments were amplified ($P < 0.05$) in samples from goats that received the transgenic soybean.

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1. Introduction

Several studies on the influence of feeding genetically modified (GM) plants on the performance of various animal species have been performed. Results have also been summarized and discussed in review articles (Aumaitre et al., 2002; Flachowsky et al., 2007, 2012). Concerning ruminants, the inclusion in the diet of ingredients derived from a wide range of GM plants did not affect feed intake, milk yield and composition. Moreover, comparable

performances in growing animals fed GM or conventional plants have been reported (EFSA, 2008).

It is well known that colostrum plays a critical role in ruminants, since its early ingestion is the one method to produce maternal antibodies (Lombardi et al., 1996). An adequate passive transfer of immunity, determined by measuring serum immunoglobulin G (IgG) concentration, is a critical determinant of the short-term health and survival of neonatal ruminants. It has been reported that, for instance, to ensure adequate passive transfer of immunity, kids should receive a sufficient volume and concentration of colostrum within the first 12 (Massimini et al., 2007) to 24 h of life (Arguello et al., 2004; Massimini et al., 2006; Mastellone et al., 2011).

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An inadequate ingestion or absorption of colostrum IgG leads to a secondary immunodeficiency condition, termed failure of passive transfer (FPT), that predisposes ruminant neonates to the development of bacterial septicaemia and common neonatal diseases (Mastellone et al., 2011). Calves with FPT have an increased risk of death until at least 10 weeks of age and, in neonatal calves <7 days old, this risk has been associated with a serum IgG concentration of <10 mg/mL (Tyler et al., 1998; Weaver et al., 2000; Barrington and Parish, 2002).

Passive transfer of immunity seems also to have a predictive value for health and productivity outcomes (i.e., subsequent growth and production) in juvenile calves, lambs, and kids both before and after weaning (Massimini et al., 2007). In dairy calves, serum IgG concentration 24–48 h after calving was a significant source of variation in average daily gain (ADG) through the first 180 days (Robison et al., 1988). Additionally, it was showed that passive transfer status was a significant source of variation in growth performance in buffalo calves. Therefore, maximizing passive transfer of immunity by allowing calves to nurse the dam can increase growth performance during the first month of life (Mastellone et al., 2011).

All these data underline the importance of passive transfer of IgG in ruminants. Therefore, the possible influence of feeding in determining the composition and quality of colostrum may be critical for the achievement of adequate passive immunity and, as a consequence, for the best growing performances. The possible effects of GM feeding in changing some protein activity and synthesis, mainly at cellular level, have been recently underlined by several researchers (Tudisco et al., 2006, 2010; Trabalza-Marinucci et al., 2008; Mastellone et al., 2011, 2013).

To current knowledge, no data are available concerning the influence of GM feeding on colostrum composition in ruminants. The aims of this study were thus to evaluate:

1. colostrum quality, in terms of chemical composition and IgG concentration, in an autochthonous goat population called 'Cilentana' bred in Cilento (Salerno province, Southern Italy), fed GM soybean;
2. in vivo and post mortem performance and serum IgG concentration in kids fed only dams milk;
3. presence of feed DNA fragments in the colostrum.

2. Materials and methods

2.1. Diets, animals and feeding

The trial was performed on a farm located at Casaleto Spartano, Salerno province, Southern Italy, at 832 m above sea level where 300 goats of an autochthonous goat population, called 'Cilentana', are bred. Two months after kidding goats generally have free access to pastures (9.00 am to 4.00 pm), constituted by 60% Leguminosae (*Trifolium alexandrinum*, *Vicia* spp.) and 40% Gramineae (*Bromus catharticus*, *Festuca arundinacea*, *Lolium perenne*).

The present experiment was performed on 40 male kids born from four groups of pluriparae goats, homogeneous in parity and milk production during the previous lactation. Sixty days before kidding, goats of each group ($n = 10$) were housed in separate sheds and fed isoenergy and iso-protein diets consisting of oat hay and commercial concentrate. The latter containing solvent extracted (s.e.) soybean meal (13% or 20% of concentrate DM) which was from conventional or GM (MON40-3-2) soybean for groups C (control; C13 and C20) and T (treated; T13 and T20), respectively.

Table 1

Chemical composition (g/kg DM) and nutritive value (UFL/kg DM) of hays and concentrates.

	Oat hay	Alfalfa hay	C13 and T13 ^a	C20 and T20 ^b
Crude protein	55.0	150.0	180	180
Ether extract	19.0	20.0	30.0	27.0
NDF	682.0	493.0	270.0	280.3
ADF	451.0	341.0	115.0	118.0
Lignin	61.0	50.0	30.0	33.0
UFL	0.47	0.72	1.03	1.00

^a Soft wheat bran 30; soybean solvent extracted (conventional or RR[®]) 13; corn meal 13; sunflower meal 10.5; citrus pulp 8; sugar beet pulp 7.9; corn gluten feed 7; sugarcane molasses 7.5; CaCO₃ 1.5; CaHPO₄ 0.7; vitamins 0.2; NaCl 0.7.

^b Soft wheat bran 31; soybean solvent extracted (conventional or RR[®]) 20; corn meal 14; citrus pulp 8; sugar beet pulp 8.9; corn gluten feed 7; sugarcane molasses 8; CaCO₃ 1.5; CaHPO₄ 0.7; vitamins 0.2; NaCl 0.7.

MON40-3-2 is tolerant to the glyphosate family of herbicides by expressing transgenic DNA from the CP4 strain of *Agrobacterium tumefaciens* (CP4 EPSPS), encoding 5-enolpyruvylshikimate-3-phosphate synthase protein (glyphosate-tolerant soybean GTS 40-3-2) (Padgett et al., 1995). A polymerase chain reaction (PCR)-end point reaction confirmed the presence of p35S and CP4 EPSPS specific transgenes (MON40-3-2) in the treated diet, as well as their absence in the control diet. Goats received oat hay ad libitum, while the concentrate was administered in amounts of 200, 300 and 400 g/head per day, 60, 30 and 15 days before kidding, respectively. After kidding, administration of the concentrate was gradually increased up to 700 g/head per day and oat hay was replaced by alfalfa hay, in order to increase the diet protein content. Animals had free access to the water. The chemical composition (Van Soest et al., 1991; AOAC, 2000) and net energy calculated according to INRA (1978) of hays and concentrates as well as ingredients of concentrate are set up in Table 1.

The trial was performed according to Animal Welfare and Good Clinical Practice (Directive 2010/63/EU) and was approved by the local Bioethics Committee. Ten male kids were randomly selected from twin deliveries in each group. Immediately after kidding kids were separated from the mothers and put into individual cages in a separate room. Each kid was fed only colostrum/milk from its mother, by using a milk feeder. Colostrum/milk was collected from each goat into sterile tubes with aseptic techniques and administered twice/day (50 mL/kg of body weight, BW), until 24 h before slaughtering.

2.2. Sampling collection and pre-treatment

Within 1 h from kidding, individual samples of colostrum were collected and divided in aliquots: (1) for IgG concentration, colostrum (10 mL) was first centrifuged at 4000 × g for 15 min to remove fat and sediments and then centrifuged at 20,000 × g for 30 min. The intermediate layer was withdrawn for analysis. (2) For DNA analysis, 10 mL colostrum was incubated at 4 °C overnight and centrifuged (2000 × g for 20 min at 4 °C) to separate the cream, skimmed colostrum and sediment; only the sediment fraction was subjected to DNA extraction. (3) No pre-treatment was performed for chemical composition (30 mL).

Twenty-four hours after kidding, blood was collected from the jugular vein of the kids. Samples were centrifuged at 2500 × g for 10 min and the serum collected for IgG concentration.

One hundred mL of milk (obtained by mixing the production of the two daily milkings) from each goat were fortnightly collected and analyzed for protein and fat content by the infrared method using a Milko Scan 133B (Foss Matic, Hillerød, Denmark), standardized for goat milk. Milk samples were collected at days 15, 30, 45, 60 after kidding.

2.3. IgG assay

IgG concentration was determined by use of a commercially available RID assay for goat IgG, according to the manufacturer's specifications. Briefly, 5 µL of serum or colostrum were added to 1 well of a 48-well plate containing anti-goat IgG antiserum, Tris-buffered saline solution, and 0.1% sodium azide in a 1.5% agarose matrix. Three reference standards (2.5, 10, and 20 mg of goat IgG/mL) included in the kit were tested concurrently with each sample. The plate was incubated at room temperature (approx

Table 2
Sequence (5'–3'), amplicon size (bp) and annealing temperature (°C) of primer pairs used in PCR.

Fragment name	Primer sequence (5'–3')	Amplicon size (bp)	T_M (°C)
Cap 144	CGC CCT CCA AAT CAA TAA G	326	55
Cap 469	AGT GTA TCA GCT GCA GTA GGG TT		
Clor1	TCCAGGGTTTCTCTGAAITTG	100	60
Clor2	TATGGCGAAATCGGTAGACG		
Le1n02-5	GCCCTACTCTACCCCCCA	118	59
Le1n02-3	GCCCATCTGCAAGCCTTTTT		
35S-1	GTCCTACAATGCCATCA	195	56
35S-2	GATAGTGGGATGTGCGTCA		
CP4EPSPS 1	GCA AAT CCT CTG GCC TTT CC	145	60
CP4EPSPS 2	CTT GCC CGT ATT GAT GAC GTC		

23 °C [73.4 °F]) for 24 h, and the precipitin ring diameter measured. The IgG concentration of test samples was determined by comparison of the precipitin ring diameter for test samples with a semilog plot generated from results for the reference standards. Samples with IgG concentration of >20 mg/mL were diluted 1:10 with sodium barbital buffer solution and reanalyzed. For statistical analyses, all samples having an IgG concentration of less than the assay's lowest detectable limit of 2.5 mg/mL, were designated as having a concentration of 0 mg/mL.

2.4. DNA extraction and quantification

Colostrum (10 mL) and, as control, conventional and transgenic soybean meal s.e. samples (100 mg) were extracted in duplicate, modifying the Wizard extraction method (Promega, Madison, WI, USA), as follows: the somatic cell pellet obtained was washed twice with phosphate buffered saline (PBS, pH 7.2). The pellet and the ground plant samples were resuspended by vortexing in 860 μ L of extraction buffer [10 mM Tris–HCl (pH 8.0), 150 mM NaCl, 2 mM EDTA, 1% (w/v) SDS], 100 μ L guanidine hydrochloride (5 M) and 40 μ L of proteinase K (20 mg/mL) and then incubated at 58 °C for at least 3 h on a shaking incubator and centrifuged at 20,000 \times g for 10 min. Five hundred μ L of the supernatant was incubated with 5 μ L RNase (10 mg/mL) at 37 °C for 10 min. One milliliter of Wizard DNA purification resin (Promega) was added to the supernatant. The DNA–resin mixture was pushed through the column and washed with 2 μ L 80% (v/v) isopropyl alcohol followed by centrifugation at 20,000 \times g for 1 min. After drying at 70 °C for 10 min, the DNA was eluted with 50 μ L of 70 °C elution buffer [10 mM Tris–HCl (pH 9.0), 0.1 mM EDTA] and centrifuged at 20,000 \times g for 1 min. The DNA was quantified with the aid of a spectrophotometer (Thermo Electron Corporation, Waltham, MA, USA), according to standard molecular techniques (Sambrook et al., 1989).

2.5. PCR analyses

In order to avoid contamination, PCRs were assembled in an ultraviolet-sterilized hood. Filter tips against sample aerosol and sterile disposable tubes were used during pipetting. The PCRs were performed in 20 μ L reaction volumes containing 20 mM/L Tris–HCl pH 8.4, 1.5 mM/L MgCl₂, 50 mM/L KCl, 100 mM/L of each dNTPs, 900 nM/L forward and reverse primers (see Table 2), 100 ng of genomic DNA templates, and 1 U of Taq polymerase (Invitrogen, Carlsbad, CA). Amplifications were performed using a Gene Amp PCR System 2400 (Applied Biosystems, Foster City, CA, USA) programmed as follows: one step of 5 min at 94 °C; 35 cycles of 30 s at 94 °C, 30 s at 60 °C (for Cap 144/496, Clor 1/2, Le1n02 5/3, 35S 1/2 and CP4 EPSPS 1/2, see Table 2), 1 min at 72 °C; and one step of 3 min at 72 °C. The PCR products were separated on 2.5% agarose gels in TBE buffer. Sequence, amplicon size and annealing temperature of the primer pair sets (Sigma–Genosys Ltd, Haverhill, UK) used for PCR, are shown in Table 2. Primers Cap 144/496 were previously used to amplify a conserved portion of caprine mtDNA, which encodes the 12S ribosomal RNA (12S rRNA) gene of mitochondrial DNA from caprine (Bottero et al., 2002). Subsequently, samples were monitored for the presence of the chloroplast sequence for tRNA Leu, by using the Clor 1/2 primers designed on the chloroplast trnL sequence (Terzi et al., 2004). Finally, species-specific primers for conventional and GM soybean were used: Le1n02 5/3 which amplifies the soybean lectin gene (Kuribara et al., 2002); 35S 1/2 and CP4

EPSPS 1/2 which amplify, respectively, part of the 35S promoter (Lipp et al., 1999) derived from the cauliflower mosaic virus and part of the specific gene sequence (CP4 EPSPS) that provides herbicide tolerance derived from *Agrobacterium tumefaciens* strain CP4 both present in the genomic structure of GM soybean (Hernandez et al., 2003). The primer pairs were selected from those reported in the literature (Jennings et al., 2003), with the aim of obtaining short amplicons (118 bp), compatible with highly fragmented DNA samples. A 100 base pairs (bp) ladder, containing linear DNA fragments, served as size standard reference. The PCR was done three times, and samples with positive results at least twice were judged as positive (Chowdhury et al., 2003). In every PCR run, positive and negative controls were included to ensure reproducibility and absence of contaminants. For positive controls, reference DNA consisting of purified conventional and transgenic soybean DNA was amplified in parallel with the samples to ensure correct performance of the PCR; for negative control (buffer blank), water instead of DNA was added to the PCR mix to check for cross contamination with soybean DNA in the PCR mix or its constituents (Klaften et al., 2004).

The amplification products (20 μ L) obtained with Clor1/2, Le1n02 5/3, 35S 1/2 and CP4 EPSPS 1/2 primers on colostrum DNA samples were separated by electrophoresis at 100 V on a 2.5% (w/v) agarose gel in 1 \times TBE buffer (89 mM Tris pH 8.4; 89 mM boric acid; 2 mM EDTA), containing ethidium bromide (10 mg/mL). The excised bands were purified following the QIAquick gel extraction kit protocol (QIAGEN GmbH, Hilden, Germany). Sequence reactions were performed using a Big Dye[®] Terminator Cycle Sequencing Kit (Applied Biosystems) and Clor1 primer. Dye terminator excess was removed using the MultiScreen separation system (Millipore Corp., Bedford, USA). Sequencing was performed using the ABI PRISM[®] 310 Genetic Analyzer (Applied Biosystems). Similarities with all sequences in the international nucleotide non-redundant data banks and with sequences from EST division were detected using the Basic Local Alignment Search Tool (BLAST) program (Altschul et al., 1997) on network servers. Amplification and sequencing were done in duplicate.

2.6. Kid body and carcass measurements

Kid body weights were taken at birth, after 30 days of age and after 24 h fasting, at slaughtering (60 \pm 7 d). After the last weight control, the following measurements were taken (ASPA, 1991): height at withers, height at pelvis, rump length, rump width, chest height, chest width, chest girth and body length. All animals were slaughtered in an authorized slaughterhouse according to EU legislation (EU Regulation EC No 882/2004). After complete bleeding, the slaughtered animals were skinned. Hot carcass, skin and certain visceral organs (abomasum, empty intestine, genitourinary tract, spleen, liver, kidney, heart and lungs) weights were recorded. In order to evaluate the carcass characteristics, the following measurements were conducted (ASPA, 1991): length of the carcass, chest width, rump width. The carcasses were weighted also after chilling for 24 h at 4 °C. Hot and cold dressing were calculated.

2.7. Statistical analyses

Data concerning colostrum and milk chemical composition, colostrum IgG concentration and kids in vivo and post mortem measurements were analyzed by ANOVA according to the following model: $y_{ijk} = \mu + A_i + B_j + (A^*B)_{ij} + \varepsilon_{ijk}$ where: y = dependent variable, μ = mean; A = diet effect (i = conventional soybean meal, groups C vs genetically modified soybean meal, groups T), B = soybean meal percentage of concentrate DM (j = 13%, 20%), A^*B = interaction diet \times soybean meal percentage and ε = error. The comparison among the mean values was performed by using the Tukey test. Correlation between IgG values of colostrum and serum were analyzed. The presence of plant DNA fragments in the colostrum was analyzed by using the χ^2 test. All the statistics were performed with SAS 2.0 software (SAS, 2000).

3. Results

Changes in the soybean content of the concentrate did not lead to statistical differences, therefore, only results concerning its genetic modification will be discussed.

Colostrum from treated groups showed a significantly ($P < 0.01$) lower percentage of protein and fat (Table 3).

Table 3

Fat and protein (%; mean ± sd) in colostrum and milk throughout the trial; IgG (mg/mL; mean ± sd) in colostrum and serum.

	C13	C20	T13	T20
Colostrum				
Fat	7.2 ± 0.3 A	7.1 ± 0.4 A	4.6 ± 0.9 B	4.6 ± 0.7 B
Protein	18.7 ± 1.4 A	18.8 ± 1.5 A	6.1 ± 0.9 B	6.0 ± 0.8 B
Milk 15 d				
Fat	3.8 ± 1.1 A	3.7 ± 1.0 A	3.0 ± 0.8 B	3.0 ± 0.9 B
Protein	4.3 ± 0.6 A	4.4 ± 0.6 A	3.8 ± 0.6 B	3.8 ± 0.6 B
Milk 30 d				
Fat	3.2 ± 0.7	3.2 ± 0.8	3.0 ± 0.9	3.1 ± 1.0
Protein	3.6 ± 0.7	3.6 ± 0.8	3.4 ± 0.9	3.4 ± 1.0
Milk 45 d				
Fat	3.2 ± 0.4	3.1 ± 0.5	3.1 ± 0.4	3.1 ± 0.5
Protein	3.3 ± 0.5	3.4 ± 0.4	3.3 ± 0.5	3.3 ± 0.4
Milk 60 d				
Fat	3.3 ± 0.4	3.3 ± 0.5	3.1 ± 0.6	3.0 ± 0.5
Protein	3.5 ± 0.4	3.5 ± 0.5	3.4 ± 0.3	3.4 ± 0.5
IgG (colostrum)	37.2 ± 4.7 A	39.0 ± 4.3 A	23.4 ± 4.1 B	22.7 ± 4.4 B
IgG (serum)	33.2 ± 5.4 A	31.2 ± 6.1 A	20.9 ± 2.0 B	18.0 ± 2.9 B

A, B: $P < 0.01$.

Similarly, both chemical parameters significantly differed in milk collected 15 days after kidding, even if such a difference disappeared in the successive samplings. Either colostrum or serum IgG concentration (mg/mL) were significantly ($P < 0.01$) lower in the treated groups (Table 3).

The quality of each DNA sample extracted from the colostrum of the control and treated goats was first verified using the Cap 144/496 primers that were used to amplify a conserved portion of caprine mtDNA 12S rRNA sequence. An example is shown in Fig. 1.

PCR was repeated three times for each sample and only samples with at least a positive result twice were judged as positive (Chowdhury et al., 2003). A chloroplast specific DNA fragment (trnL, 100 bp) was found in the majority ($P < 0.01$) of the control and treated samples. Several control and treated samples were found positive ($P < 0.01$) for the lectin gene fragments. Transgenic target DNA sequences (35S and CP4 EPSPS) were not detected in colostrum from control goats that received a diet containing conventional soybean meal. By contrast, transgenic DNA fragments were

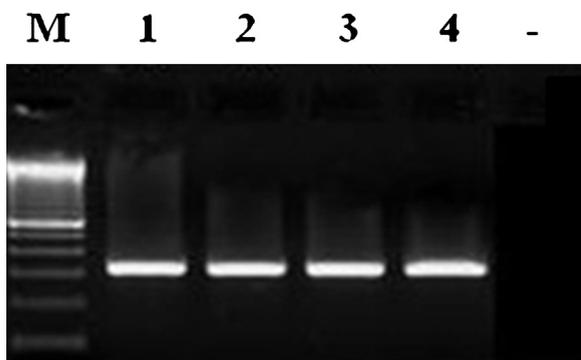


Fig. 1. Representative electrophoresis gels of amplified DNA in colostrum from C13 and C20 (lines 1 and 2, respectively) and T13 and T20 (lines 3 and 4, respectively) goats. Lane M contains a 100 bp DNA ladder; '-' is a negative control (no DNA template).

Table 4

Number of animals in which DNA sequences were detected.

	Chlor	Lectin	35S	CP4 epsps
C13	8/10**	6/8**	0/8	0/8
C20	8/10**	6/8**	0/8	0/8
T13	8/10**	5/8**	5/8*	5/8*
T20	8/10**	6/8**	5/8*	5/8*

The lectin, 35S and CP4 EPSPS fragments were investigated only in those samples, which were positive for the chloroplast DNA fragment (Chlor).

* $P < 0.05$.

** $P < 0.01$.

amplified ($P < 0.05$) from samples from goats that received transgenic soybean (Table 4).

In Fig. 2, a representative example is demonstrated illustrating the detection of endogenous high copy chloroplast specific DNA fragment (trnL, 100 bp) in goat colostrum of all the goats groups. A representative example obtained by using Le1n02 5/3 soybean specific primers shows the lectin signal detected in goats colostrum samples and in the plant control, as well as in control and treated groups of kids. Transgenic target DNA sequences (35S and CP4 EPSPS) detected in goats that received a diet containing transgenic soybean meal, as well as in the RoundUp Ready soybean positive control represented in Fig. 3.

The body measurements are reported in Table 5. Kid body weights were similar at birth, while they were significantly ($P < 0.05$) higher in control groups at day 30 and at slaughtering. In addition, kids from mothers fed conventional soybean showed significant ($P < 0.05$) higher height at the withers and chest width. Concerning the post mortem measurements (Table 6), significant differences were found only for the carcass weight ($P < 0.05$), which was lower in kids from the treated group.

4. Discussion

As seen, fat and protein content in colostrum and milk until 15 days after kidding was lower in the GM group, and this difference disappeared at the successive milk samplings. There is no available explanation that could support the effect of GM feed on milk fat and protein whereas for colostrum protein the results could be attributed to the lower IgG concentration in the treated group.

Presumably, these last results could be due to a reduction of B population when animals fed GM diet, as reported by Finamore et al. (2008) in mouse. Also Walsh et al. (2011) and Buzoianu et al. (2012) recorded a reduction of B lymphocyte in sows fed GM maize even if in the last case the differences were not significant. Walsh et al. (2011) also found a higher production of Interleukin-4 (IL-4) from cultured ileal lymphocytes in GM maize-fed pigs. According to Murtaugh et al. (2009), IL-4 has an antiproliferative effect on B-cells and this may account to the observed reduction. Previously, Rincon et al. (1997) had found that both IL-6 and IL-4 play a major role in mediating B-cell activation and antibody production.

The concentration of colostrum and kids serum IgG were correlated in all the groups ($r = 0.697, 0.829, 0.744$ and 0.729 , for C13, C20, T13 and T20, respectively, $P < 0.05$), according to Chen et al. (1999) who recorded significant

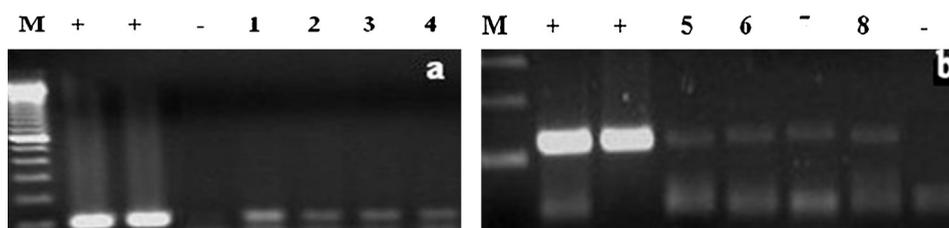


Fig. 2. Endogenous chloroplast specific DNA fragments, trnL (a) and lectin gene (b) in colostrum from C13 and C20 (lines 1 and 2, lines 5 and 6, respectively) and T13 and T20 (lines 3 and 4, lines 7 and 8, respectively) goats. M: 100 bp DNA ladder; ‘-’: negative control, ‘+’: positive control.

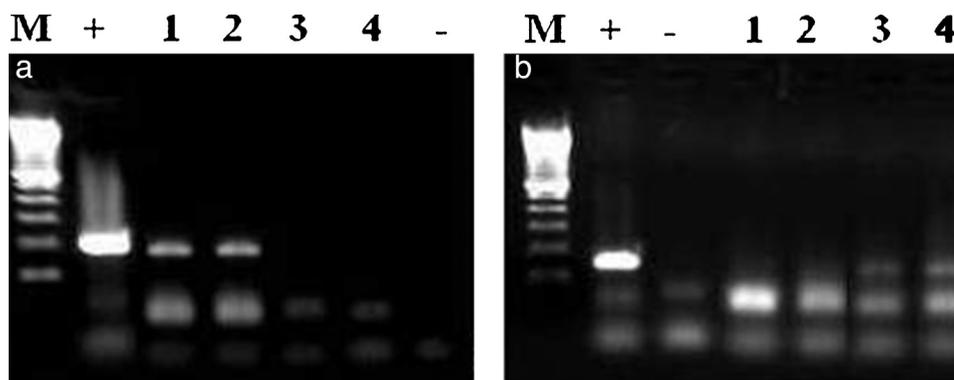


Fig. 3. Transgenic DNA fragments: (A) 35S promoter in colostrum from C13 and C20 (lines 3 and 4, respectively) and T13 and T20 (lines 1 and 2, respectively) groups, (B) CP4 EPSPS gene in colostrum from C13 and C20 (lines 1 and 2, respectively) and T13 and T20 (lines 3 and 4, respectively) groups. M: 100 bp DNA ladder; ‘-’: negative control, ‘+’: positive control.

Table 5

Mean (\pm sd) kid body weight (kg) at birth, after 30 days and at slaughtering and body measurements (cm).

	C13	C20	T13	T20
Body weight at birth	3.8 \pm 0.48	3.7 \pm 0.47	3.7 \pm 0.45	3.8 \pm 0.42
Body weight after 30 days	9.5 \pm 0.4 a	9.4 \pm 0.5 a	8.3 \pm 0.7 b	8.2 \pm 0.6 b
Body weight at slaughtering	12.5 \pm 0.4 a	12.3 \pm 0.5 a	10.3 \pm 0.5 b	10.1 \pm 0.6 b
Height at withers	48.2 \pm 2.6 a	48.8 \pm 2.4 a	45.7 \pm 2.1 b	46.0 \pm 2.0 b
Height at pelvis	47.7 \pm 2.4	47.9 \pm 2.5	45.3 \pm 1.9	45.5 \pm 2.0
Rump length	15.3 \pm 3.7	15.2 \pm 3.5	15.8 \pm 2.6	15.7 \pm 2.5
Rump width	13.9 \pm 1.4	14.1 \pm 1.5	13.3 \pm 1.9	13.5 \pm 2.0
Chest height	18.7 \pm 4.5	18.9 \pm 4.5	19.1 \pm 3.9	19.2 \pm 3.5
Chest width	12.6 \pm 1.03 a	12.7 \pm 1.1 a	11.3 \pm 1.0 b	11.5 \pm 1.1 b
Chest girth	52.7 \pm 4.6	52.9 \pm 4.5	52.2 \pm 2.7	52.1 \pm 2.5
Body length	42.0 \pm 5.6	42.5 \pm 5.5	41.8 \pm 4.9	42.0 \pm 4.5

a, b: $P < 0.05$.

Table 6

Mean (\pm sd) kid carcasses and organs weight (kg) dressing percentage (%) and post-mortem measurements (cm).

	C13	C20	T13	T20
Slaughtering weight	12.5 \pm 0.4 a	12.3 \pm 0.5 a	10.3 \pm 0.5 b	10.1 \pm 0.6 b
Hot carcass weight	7.2 \pm 0.4 a	7.1 \pm 0.5 a	6.4 \pm 0.3 b	6.2 \pm 0.4 b
Cold carcass weight	6.9 \pm 0.7	6.8 \pm 0.6	6.2 \pm 0.4	6.0 \pm 0.4
Skin	1.2 \pm 0.07	1.2 \pm 0.08	1.0 \pm 0.06	1.1 \pm 0.05
Abomasum	0.4 \pm 0.09	0.5 \pm 0.08	0.3 \pm 0.06	0.4 \pm 0.06
Empty intestine	0.8 \pm 0.23	0.7 \pm 0.25	0.6 \pm 0.08	0.5 \pm 0.07
Genito-urinary tract	0.03 \pm 0.01	0.03 \pm 0.01	0.03 \pm 0.004	0.03 \pm 0.006
Spleen	0.03 \pm 0.005	0.03 \pm 0.006	0.03 \pm 0.006	0.03 \pm 0.005
Liver	0.3 \pm 0.06	0.4 \pm 0.06	0.3 \pm 0.01	0.3 \pm 0.01
Kidney	0.07 \pm 0.01	0.08 \pm 0.02	0.06 \pm 0.01	0.07 \pm 0.01
Heart	0.1 \pm 0.05	0.1 \pm 0.05	0.07 \pm 0.01	0.06 \pm 0.02
Lungs	0.3 \pm 0.06	0.4 \pm 0.05	0.3 \pm 0.03	0.4 \pm 0.03
Hot dressing (%)	57.6 \pm 0.3	57.7 \pm 0.4	62.1 \pm 0.3	61.4 \pm 0.4
Cold dressing (%)	55.2 \pm 0.4	55.3 \pm 0.4	60.2 \pm 0.3	59.4 \pm 0.4
Length of carcass	43.0 \pm 1.9	43.1 \pm 2.0	41.5 \pm 2.1	41.6 \pm 2.3
Rump width	12.0 \pm 0.5	12.2 \pm 0.6	10.8 \pm 1.1	11.0 \pm 1.2
Chest width	11.2 \pm 2.2	11.0 \pm 2.0	10.1 \pm 2.0	10.2 \pm 2.5

a, b: $P < 0.05$.

differences in serum IgG concentrations between kids fed high or low protein concentration colostrum (20 and 10 g/dL, respectively). [Stott and Fellah \(1983\)](#) reported that the concentration of colostrum IgG is a determinant factor in the absorption of IgG by calves. Animals which consume 1 L of colostrum with a concentration of 60 mg/mL showed a higher concentration on serum IgG, compared to those which consumed 2 liters of colostrum with a concentration of 30 mg/mL. Similarly, [Muller and Ellinger \(1979\)](#) reported positive correlation coefficients between the concentration of IgG (0.52), IgA (0.56) and IgM (0.36) in the colostrum and blood in calves. In contrast, [Dos Santos et al. \(1994\)](#) found no relationship between colostrum and serum IgG concentration in one day-old kids, while [Al-Jawad and Lees \(1985\)](#) did not register a different absorption efficiency in lambs fed with different sources of IgG.

Kid body weights were similar at birth, while they were significantly higher in control group at day 30 and at slaughtering. The potential effects of low quality colostrum (low IgG content) may be critical in terms of both kid's survival and growth. A significant association was detected between serum IgG concentration 24 h after birth and day-30 weight and between serum IgG concentration 24 h after birth and ADG from birth to day 30 in lambs ([Massimini et al., 2006](#)) and buffalo calves ([Mastellone et al., 2011](#)). These results suggest that passive transfer status, determined as serum IgG concentration 24 h after birth, was a significant source of variation in growth performance during the first month for newborn ruminants nursed by the dam.

Since colostrum IgG represents the one source of antibodies for kids, results surely lead to a reduction of passive transfer status, that may affect growth performances. Indeed, immunoglobulin in colostrum are highly related to a number of growth and maturation factors, such as somatomedins (IGF-1, IGF-2), somatotrophin, fibroblast growth factor, transforming growth factor (TGF), insulin, platelet derived growth factor (PDGF) and epidermal growth factor (EGF), which form a powerful combination ([Oda et al., 1989](#); [Ginjala and Pakkanen, 1998](#)). These naturally occurring substances have been shown to enhance the synthesis of DNA, RNA and protein, while at the same time inhibiting the breakdown of protein ([Ballard et al., 1982](#); [Ginjala and Pakkanen, 1998](#)). There are receptors for these compounds throughout the intestinal tract, and they are postulated to be mediators of intestinal growth and development ([Montaner et al., 1999](#)). Maturation and proliferation of the intestinal cells resulted in increased absorption of electrolytes and nutrients from intestine ([Opleta-Madsen et al., 1991](#); [Alexander and Carey, 1999](#)).

In this study kids of goats fed GM soybean showed lower weights at slaughtering, despite there being no differences in milk composition between groups in the last sampling. Therefore, no compensatory growth was observed.

Transgenic fragments were detected in colostrum of the treated groups. These findings show plant DNA fragments are likely to survive the digestive processes ([Duggan et al., 2003](#); [Einspanier et al., 2004](#)). Recombinant plant DNA has also been found by other researchers to be a rare event ([Chowdhury et al., 2003](#); [Mazza et al., 2005](#); [Sharma et al., 2006](#)) in organs and tissues of animals fed GM feeds. Studies

aiming at verifying the presence of transgenic fragments in milk gave contrasting results. In dairy cows fed GM soybean meal and maize ([Phipps et al., 2003](#); [Nemeth et al., 2004](#)) or goats fed GM maize ([Rizzi et al., 2008](#)), the chloroplast DNA fragments were detected in milk, but no transgenic fragments were found. Instead, [Tudisco et al. \(2010\)](#) detected fragments of *epsps* gene and 35S promoter in milk of goat fed RR soybean and significant modifications of LDH activity in liver, kidney and heart of their kids fed only dams milk. Further studies need to verify the possible role of transgenic DNA fragments found in colostrum of treated groups in this trial on the results above depicted.

5. Conclusions

A significant decrease in growth performances of kids from mothers fed genetically modified soybean, either 13 or 20% of the concentrate DM, was observed. The growth delay started immediately after kidding and was presumably due to the lower percentage of protein in the colostrum as well as in the milk at 15 days of lactation. In addition, the lower concentration of IgG highly linked to a number of growth and maturation factors could have contributed to the results. Further research is needed to understand the role played by the transgenic fragments detected in the colostrum.

Conflict of interest

None.

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